ALTERNARIA LEAFSPOT DISEASE OF CUCURBITS

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INTRODUCTION

Alternaria leafspot disease of cucurbits, caused by Alternaria cucumerina (E. & E.) J. A. Elliot, is an important disease in the United States. During the past 2 years, observations have been made in the vicinity of Gainesville, Florida, and data have been collected concerning the nature of the pathogen and its relation to various cucurbit hosts. Incomplete information concerning the disease is found in the literature and does not lead to a clear understanding of the disease or its causal agent. This paper presents a detailed discussion of the disease, the pathogen in artificial culture, and its relation with the host as found in Florida.

THE DISEASE

One of the first references to Alternaria leafspot of cucurbits was made by Berlese (3) in 1892 when he briefly described a disease of melons which had been found in Italy. The disease was present in New Mexico as early as 1895 (16), and a year later Sturgis (58) reported a similar disease of muskmelons in Connecticut. It also appeared in Ohio in 1896 as reported by Selby (55). Hume (23) was apparently the first to report the disease from Florida in 1901, and during the succeeding 15 years reports were made from most of the southeastern and southwestern states.

The disease has been known by several frequently used common names in the United States such as rust, leaf blight, cantaloupe blight, target leaf spot, Alternaria leafspot, Alternaria blight, and Macrosporium blight. Many of these names as applied to the disease are inappropriate and confusing. Since the characteristic disease symptoms appear on the leaves of the host, the writer prefers the name Alternaria leafspot and this name will be used in the text.

Host Range

Alternaria leafspot disease has been reported to occur on cucurbits under natural conditions. The most frequently reported host is muskmelon, principally the cantaloupe, Cucumis melo var. cantalupensis Naud., and the cassaba or honey dew melon, C. melo var. inodorus Naud. Destructive outbreaks of the disease on watermelon, Citrullus vulgaris Schrad. and cucumber, Cucumis sativus L., have been reported by a number of investigators but, judging from the literature, the occurrence of severe widespread infection of these hosts is relatively infrequent. The pathogen has been

reported to attack squash, but usually such reports have not specified the species of host involved. Weiss (66), in a compilation of reported plant diseases in the United States, lists Cucurbita maxima Duchesne, C. moschata Duchesne, C. pepo L., and C. pepo var. melopepo Alef. as hosts. Burger (8) found the disease on the balsam pear, Momordica charantia L.

Brisley (7) extended the host range by artificially inoculating tomato and potato, although symptoms on these hosts were not given. Young (68), using a culture of the pathogen supplied by Brisley, obtained infection of wheat seedlings growing under sterile conditions in a test tube and corn leaves under greenhouse conditions. Artificial inoculation of plants in the greenhouse during this study has extended the host range to include Cucurbita pepo var. ovifera Alef., yellow-flowered gourds; Cucumis anguria L., bur gherkin; Momordica balsamina L., balsam apple; and Melothria pendula L. Details of these inoculations will be discussed under pathogenicity tests.

Geographical Distribution

Many members of the genera Citrullus and Cucumis are of African origin according to Bailey (1) and one of the first reports of the disease on muskmelon was made by Berlese (3) in Italy, followed shortly by a series of reports by Peglion (47, 48, 49) in Italy. Nicolas (44) in France and Wardlaw et al. (62) in Trinidad described fruit rot symptoms attributed to the pathogen. The presence of the disease in Egypt was reported by Melchers (36), and it was reported by Synnatt (59) to be present in New South Wales. The disease has been found in Chile (40) and Venezuela (50).

The occurrence of Alternaria leafspot is most prevalent in the United States, where the disease has been reported in 33 states. A report (21) from Michigan assessed the loss in one year as 90 per cent of the

muskmelon crop. Barrus et al. (2) reported the disease from Wisconsin, Massachusetts, and North Caroline, while Blain (4) found the disease in Alabama. Brisley (7) concluded that the disease was of great importance in Arizona, and it had been reported previously in neighboring states by Smith and Smith (56) in California, Heald and Wolf (22) in Texas, and Ellis and Everhart (16) in New Mexico. In a compilation of plant disease reports, McMillan (34) listed the disease as being found in New York, New Jersey, Delaware, Maryland, Arkansas, Georgia, Florida, Illinois, Colorado, Utah, and Oregon. Gregory (19) in Indiana, Selby (55) in Ohio, and Edson (14) in Iowa have reported Alternaria leafspot from these midwestern states. According to Weiss (66), the disease has been found in New Hampshire, Minnesota, West Virginia, and Pennsylvania. In a recent report concerning important cucurbit diseases in South Caroline, Epps (17) mentioned the occurrence of Alternaria leafspot, and Miller (39) found it to be present in Tennessee. Cook (10) reported the disease from Virginia, and Sturgis (58) in Connecticut found it on muskmelons as early as 1895. In a survey of plant diseases in Oklahoma, Larsh (28) found slight infaction of cucurbits caused by the pathogen and KenKnight and Blodgett (26) gave a similar report from Idaho. The states from which the diseasa has not been reported are primarily in the north central and northwestern sections of the United States, but it seems probable that the disease is present in every state where cucurbits are grown commercially.

Economic Importance

The losses from Alternaria leafspot vary greatly from year to year within its host range. Muskmelon losses occur most consistently since this crop is the principle host of the pathogen. Blinn (5), in 1905, was the first to call attention to the serious problem facing growers in regions

of Colorado where muskmelons constituted a principal annual commercial crop. Stevens and Hall (57) listed the disease as the most destructive found on muskmelon in the United States. Brisley (7) considered the disease of great economic importance in Arizona, citing the complete loss of the 1921 crop in some sections. Muskmelon losses in southwestern Indiana have been reported frequently and Hartman and Gaylord (20) considered Alternaria leafspot as one of the most destructive diseases. LeClerg (29) reported an outbreak in Colorado which caused considerable losses of muskmelons. Lindford (31) reported the disease to be severe in Green River, Utah, where losses reached 30 per cent in one field with all leaves infected and 20 per cent defoliation. The disease appeared yearly in Georgia from 1925 to 1932, and in 1925 and 1927 it was very severe according to Van Haltern (61). Godfrey (18) observed the disease in epiphytotic proportions in Texas. In Florida, since Hume's report in 1901 (23), the disease has been reported frequently as causing varying amounts of damage.

Watermelon and cucumber losses have rarely been estimated in reports of the occurrence of the disease on these hosts. According to Orton (45) and Parris (46), the disease is usually insignificant on watermelon. Weber and Owen (65) reported up to 75 per cent infection in some of the 11 fields they surveyed in central Florida. McWhorter (35) found the disease prevalent on cucumber foliage in Oregon, attacking immature plants and continuing as a mild leaf spot throughout the growing season. The disease on squash is apparently of little importance as reports concerning this host are not numerous and give no indication of the extent of the infections. Reports in the literature are of small value in attempting to assess the monetary losses caused by Alternaria leafspot. No data

are available concerning losses sustained as a result of leaf infection. Wiant (67) gives some indication of market spoilage of muskmelons due to Alternaria spp., but interpretation of these losses is difficult since several species are involved.

Symptoms

Descriptions of Alternaria leafspot symptoms on cucurbit leaves have been given by Parris (46), Hume (24), Weber (63), Brisley (7), Martin (33), Peglion (47), and Stevens and Hall (57), but they are rather brief and incomplete. In addition there are certain distinct differences in the development and appearance of leaf symptoms on various hosts. The symptoms observed on different varieties of muskmelons are generally quite similar, varying mostly with respect to rate of development and number of lesions. The same observation applies for watermelon, cucumber, and squash varieties. The symptoms of each of these hosts, therefore, must be discussed separately. Fruit symptoms incited by the Alternaria leafspot pathogen have been reported occasionally on cucurbits and without exception have been noted on fruits in market or storage, or on overripe or damaged fruits in the field. Wardlaw et al. (62) described a cucumber storage rot from Trinidad. Tyler (60) reported a destructive storage rot of acorn squash. Gregory (19), Brisley (7), and LeClerg (30) observed infection of overripe muskmelons associated with leaf symptoms in the field, while Nicolas (44) described symptoms which developed in French markets. Nattrass (41) reported that a ripe rot of watermelon was caused by the pathogen. Weber (63) pictured a cucumber with a dark surface growth of the pathogen. Stem and petiole symptoms have not been reported commonly. Brisley (7), in his study of the disease, mentions the absence of symptoms on these organs. The writer has never seen symptoms on stems, petioles, flower parts, or

roots of any cucurbit.

Muskmelon Symptoms

The earliest leaf symptoms appear as small yellow or brown flecks on the adaxial surface of the blade. These flecks are 0.5-1.5 mm in diameter, irregular to circular and surrounded by a faintly chlorotic halo. Upon close scrutiny these flecks appear as minute shallow craters with thin, whitish, translucent central convexities and light brown ridges or shoulders. A water-soaked grayish furrow delimits the lesion. The tissue surrounding the lesion is pale green to yellow, 3-8 mm in diameter, and halo-like with indefinite margins (Fig. 1). At this initial stage of the development of the disease the abaxial surface symptoms are characteristic, small, pale flecks which correspond to lesions on the adaxial surface. The flecks, which are quite membranous in appearance, are in the centers of small, shallow, gray, water-soaked invaginations. The chlorotic halo is apparent on the adaxial surface in most cases, but occasionally it fails to develop. The extent of the halo varies greatly and on highly susceptible varieties chlorosis may extend out as much as 5 mm from the point of infection with the chlorotic tissue becoming distinctly blister-like. If environmental conditions are favorable, lesions enlarge rather rapidly; during unfavorable ecological conditions the chlorotic blister may remain essentially unchanged for many days or the necrotic fleck may enlarge very slowly and assume a uniform color and shape.

Necrosis of tissue proceeds from the necrotic fleck outward in a roughly equi-radial manner, developing a circular to somewhat irregular lesion and becoming brown in the center and lighter colored at the edges. A slightly raised ridge delimits the necrotic tissue, with a distinct, discontinuous furrow between the ridge and the outer chlorotic tissue.

Fig. 1.--Symptoms on the adaxial surface of Rio Gold cantaloupe leaf 6 days after inoculation. X 2.

The lesion may under certain conditions develop distinct, roughly concentric zones which give a characteristic "target board" appearance to the lesion. The development of this zonation is not a constant feature of lesion expansion. The outer ridge which delimits the lesion is darker brown than the centripetally adjacent tissue and as growth of the pathogen involves more tissue, necrosis of adjacent tissue forms the ridge between 2 bands of lighter colored tissue. The collective effect of all dark ridges and lighter colored intervening bands is observed as a distinct zonation pattern. When first formed the ridges are barely raised above the lesion surface, and as the lesion enlarges the ridges tend to become depressed, being distinguished later only by a darker brown color. The reasons for the presence or absence of zones are not known completely. In some instances lesions which have developed in the greenhouse have exhibited strong zonation and have been studied closely. In a few cases the zones are associated with light fluctuations, the lighter tissue being developed during the dark hours, and the darker tissue corresponding to daylight hours. No more than 1 dark and 1 light zone was noted to correspond to a 24 hour period. The zones are not of value as indicators of age of the infection.

As the disease progresses, the tissues of the lesion tend to become more uniformly dark brown and slightly sunken below the leaf surface. The chlorotic halo decreases slightly in width until, at the time of maximum lesion size, its width is 0.2 to 1.0 times the diameter of the necrotic tissue. Coalescence of lesions is very common and leads to irregular necrotic patches which may be of considerable extent. The size of the fully developed lesion is quite variable, depending in part on the environmental conditions during the progress of the disease and the susceptibility of the host. Individual discrete lesions are generally 5-20 mm

in diameter but are occasionally found to be only 1-2 mm in diameter (Fig. 2). On the abaxial surface the lesion appears very similar except that zonation, if present, is not apparent and the necrotic tissue is somewhat lighter in color.

Marginal necrosis of leaf blades is a very common symptom and varies slightly from the symptoms thus far described. Lesions which occur initially along the leaf margin tend to develop rapidly and often do not advance with an equi-radial margin. Many times these lesions develop irregularly and progress inwardly with a wedge-shaped infection margin. Hume (24) and others have commented upon the disintegration of the central part of the lesion during the late stages of the disease. This breaking away of the lesion rarely, however, approaches a shot-hole condition. According to Cunningham (11), no cicatrix is formed during the lesion development and regular perfoliations do not develop. As the lesions dry they tend to crack slightly, and eventually portions of the dry tissue fall away, leaving ragged holes. Marginal lesions often become laciniate or longitudinally torn.

The time at which sporulation occurs is variable. Conidiophores form initially around the central necrotic fleck and later over the entire surface of the lesion, but they do not develop closer than 1 mm to the margin of the lesion. If the lesion is zonate, many conidiophores form on the darker tissue of the ridges and, by their presence, enhance the "target board" appearance. Conidia occur on both surfaces of the lesion but predominate on the adaxial surface. Conidia are fugacious and sporulating lesions may have many detached conidia lying on the surface. An abundance of conidia gives the lesion a dark, sooty appearance.

When leaves are heavily infected, chlorosis becomes general during

Fig. 2.--Advanced symptoms on the adaxial surface of Rio Gold cantaloupe leaf. Discrete, marginal, and confluent lesions are shown. X 4/5.

the late stages of the disease and lesions become dry and brittle. Leaves begin to curl along the margins and this curling and accompanying dessication continues until the leaves are entirely dead and fall from the vines. In the field the foliage gradually becomes less abundant until only a few leaves remain and the stems and fruits are fully exposed (Fig. 3). The effect of the early death of many leaves is dependent upon the stage of host development at the time of infection. The effect may be negligible if the fruits are close to maturity, but infection occurring at the time of fruit set or slightly later will result in reduced quality and quantity of fruit as discussed by Blinn (5). Muskmelon fruit symptoms appear as irregular, confluent, shallow depressions which may be several centimeters in extent and covered with a black-olivaceous mat consisting of mycelium, conidiophores, and conidia (Fig. 4).

Watermelon Symptoms

Leaf blade symptoms begin with the appearance of small tan flecks on the adaxial surface which, upon close inspection, are found to be 0.2-0.5 mm in diameter and saucer-like in shape with thin, whitish centers and light brown edges. A pale green to bright yellow chlorotic halo, 1-2 mm in diameter, surrounds each fleck. The lesion develops similarly to that of muskmelon in regard to ridge formation and presence of alternating bands of color. The development of the infection is rather slow and lesions usually become dark brown to black and slightly depressed while still less than 2 mm in diameter (Fig. 5). As the lesion enlarges, the chlorotic halo becomes very narrow or indistinct and zonation is frequently noted. Lesions are 1-10 mm in diameter, generally circular, and may become confluent, forming extensive necrotic areas. On the abaxial surface the initial symptoms are indistinct, but as the area of necrosis enlarges the

Fig. 3.--Defoliated muskmelon planting showing exposed condition of stems and fruits.

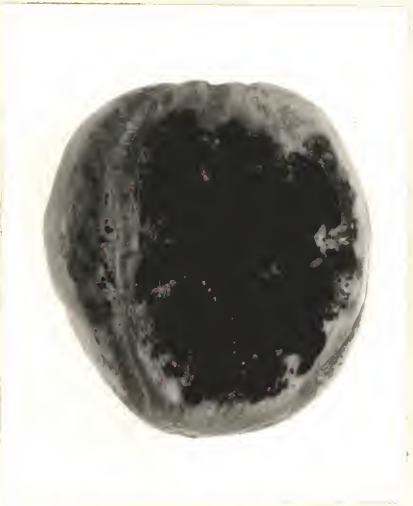

Fig. 4.--Alternaria leafspot pathogen growing over the surface of a sun-scalded area of muskmelon fruit.

Fig. 5.--Symptoms on the adaxial surface of Black Diamond watermelon leaf 14 days after artificial inoculation. X 1/2.

lesion becomes visible as a dull brown, non-zonate spot.

The symptoms of Alternaria leafspot can readily be confused with those of anthracnose and gummy stem blight, and in some cases only the presence of numerous conidia of the pathogen distinguishes it. Conidia are borne on both surfaces of the lesion, scattered on the lower surface and predominantly on the dark bands of the upper surface. A severe widespread infection of watermelon has not been observed during the course of this study. The disease has been observed most often on the older leaves around the centers of hills and at times associated with other diseases. Fruit infection has not been observed by the writer. The report of Nattrass (41), who found a ripe rot of watermelon, apparently is unique in this respect.

Cucumber Symptoms

On the adaxial surface of the leaf blade, the earliest symptom on cucumber and bur gherkin is a small bright to pale yellow spot, 1.0-1.5 mm in diameter and circular or nearly so. With considerable magnification a small, whitish, depressed fleck about 0.5 mm in diameter can be discerned in the center of the chlorotic tissue. The chlorotic spot and depression are not initially visible on the abaxial surface, but later a whitish, water-soaked depression becomes visible. Development of the disease is very slow compared with muskmelon. As the lesion expands the necrotic tissue becomes light brown and slightly depressed with no ridge formation or zonation evident. The older necrotic tissue around the point of penetration may become slightly raised and shield-like. A feature that is usually found is the darkening of the veinlets in the lesion area so that the lesion has a netted appearance. Lesions attain a diameter of 1-12 mm and are nearly circular, lobed, or otherwise irregular (Fig. 6). Conidia

Fig. 6.--Symptoms on the adaxial surface of Marketer cucumber leaf 20 days after inoculation. X 1/2.

are borne on both surfaces of the lesion, being more abundant on the adaxial surface. When conidia are very numerous they give the lesion a dark, sooty appearance. Naturally occurring infection has not been observed by the writer.

Fruit symptoms have been observed on sun-scalded cucumbers in the field and are essentially the same as those on muskmelon fruit (Fig. 7). Wardlaw et al. (62) reported that the pathogen caused a rotting of the stem end and also superficial spreading spots which became involuted and covered with the mycelium and conidia.

Squash Symptoms

The three species and various varieties of Cucurbita, into which common squashes are grouped, exhibit very similar symptoms and will be discussed collectively. Initial leaf symptoms are seen on the adaxial surface as sunken, whitish, irregular flecks, 0.5-1.0 mm in diameter with pale yellow halos 1.0-1.5 mm in diameter. On the abaxial surface the initial fleck is barely visible. The disease progresses rapidly under favorable conditions and the chlorotic areas enlarge and frequently become blister-like. On leaves having numerous infections, confluence of the chlorotic areas is common and is soon followed by general chlorosis of the entire leaf. The development of necrotic tissue, following the chlorotic stage, is centrifugal from the central fleck. Discrete lesions are tan to brown, membranous, slightly sunken, 1-12 mm in diameter, and often have a dark brown border. Veinlets tend to become dark in the necrotic areas but are not characteristically outstanding. Marginal necrosis is common during the late stages of the disease, in some cases resulting principally from the confluence of numerous infections near the leaf margins (Fig. 8). Sporulation occurs over both surfaces of the lesion but is more abundant on the adaxial surface.

Fig. 7.--Alternaria leafspot pathogen growing over sun-scalded areas on cucumbers.

Fig. 8.--Symptoms on the adaxial surface of Coccozelle Bush squash leaf 14 days after inoculation. X 1/4.

CAUSAL ORGANISM

Taxonomy

Several species of Alternaria and Macrosporium have been described from cucurbitaceous material. Some of these species are not morphologically similar to the leaf spot pathogen, and species that have been described from picked or decaying fruits are perhaps of doubtful pathogenicity, since they were found in a habitat favorable for many saprophytes. Macrosporium nitens f. colocynthidis Trotter, M. granulosum Bubak, M. lurisicii Ranojevic, M. peponicolum Rabenh., and M. lagenariae Thum. are representative of these fungi which will not be considered further.

Four fungi have been reported to cause leaf spots of various cucurbits. Alternaria plurisepata (Karst. & Har. ex Peck) Jorstad has been reported (25) to incite a leaf spot disease of cucumber and vegetable marrow. Neergaard (42) referred to the fungus as it appeared on cucumber as Stemphylium ilicis Tengwall and discussed the spore characteristics as seen on various hosts. The Alternaria-like conidia which may be formed are short beaked; the short length of the conidial body and other morphological features are distinctly different from those of the pathogen which causes the Alternaria leafspot disease of cucurbits.

Alternaria cucurbitae Letendre and Roumeguere has been cited occasionally as the cause of a leafspot disease of various cucurbits. Boughey (6) reported it on vegetable marrow in Anglo Egyptian Sudan and Schwarze (54) listed it among the fungi found in New Jersey. The latter report is questionable. Schwarze illustrated a conidium of A. cucurbitae, but the width of the conidial body was approximately the same as that shown for Macrosporium cucumerinum E. & E. Saccardo (52) gave the width

of A. cucurbitae as 8-9μ, which is distinctly narrower than M. cucumerinum. Berlese (3) commented on the pathogen causing muskmelon leaf symptoms, which was later named A. brassicae f. nigrescens by Peglion (47). He believed that the larger spores of this form could not be identical with A. cucurbitae. Peglion (47) believed A. cucurbitae to be distinct from A. brassicae f. nigrescens principally on the basis of the great difference in spore body diameter. The 2 species apparently are distinct, but considerable confusion has resulted from their similarity. Elliot (15), in his study of the genus, did not include A. cucurbitae because he was unable to obtain sufficient material. Although A. cucurbitae should be regarded as a valid species, it is not the causal agent of the Alternaria leafspot disease of cucurbits.

In 1893, Peglion (47) described A. brassicae f. nigrescens as follows:

> Hyphis brevibus, continuis, brevissime ramulosis apice aequalibus, coespitulosis; conidiis super impostis cito deciduis, fusoideo-clavatis 60-80≈14-18, initio continuis, tandem 6-8 septato-muriformibus, brunneis vel fuligineis. Habitat in maculis aridis foliorum Cucumis Melonis prope Avellino. Ab A. Brassicae differt colore conidiorum.

In the text of the article in which the form was described, he gave conidia size as 60-85 x 15-20μ and mentioned the characteristic elongated apex which could be confused with the conidiophore. Despite these comments, the formal diagnosis did not mention the beaks and gave the conidia shape incorrectly. In a second paper concerning the same fungus, Peglion (48) called attention to an error in his original description of the spore dimensions. The revised dimensions were given as 100-160 x 14-20μ, reaching 200μ in length when the filiform tip was greatly elongated. This revision apparently indicated that the original description was based only on spore body length and that the revised length range resulted from inclusion of the beaks in the measurements. In a later paper, Peglion (49) gave a

Latin diagnosis which was almost identical with the original diagnosis except that the conidia dimensions agreed with his later revision. Nicolas (44) measured conidia of a species which he believed to be A. brassicae f. nigrescens and gave dimensions which substantially agreed with those in Peglion's later papers (48, 49). Saccardo (53) listed the fungus as a variety instead of a form as originally described and, apparently following his concept, this category has been used subsequently almost without exception. A clearer idea of the fungus can be obtained from measurements which Neergaard (42) made. He measured 10 conidia which were taken from the type material and found the conidial body to average 72.6 x 16.9μ. Lengths of the conidia including beaks were 82-240μ.

Macrosporium cucumerinum was described by Ellis and Everhart (16) in 1895 from infected muskmelon leaves. Their description is as follows:

> On living leaves of Cucumis melo, Las Cruces, New Mexico, August 1894. Prof. E. O. Wooton. Epiphyllous, on orbicular, subconfluent, rusty-brown spots, 3-4 mm diam., becoming whitish in the center. Hyphae fasciculate or solitary, few in a fascicle, subgeniculate, 1-3 septate, 30-50 x 5-6μ. Conidia clavate, slender-stipitate, 3-8 septate, scarcely constricted, submuriform, 30-75 x 15-25μ, pedicel, 25-35μ long. Nearly allied to Macrosporium solani E. & M., but differs in its slender pedicellate, mostly smaller conidia.

The authors made a clear distinction between body and beak but mistook the latter for pedicels and gave the spore form as clavate. The names M. cucumerinum E. & E. and A. brassicae var. nigrescens Pegl. have been used frequently in the literature when reference was made to a causal organism of a leaf spotting disease of cucurbits, but a distinction between the symptoms was not clearly drawn. In 1917, Elliot (15), in his taxonomic study of Alternaria and Macrosporium, proposed an emendation to the circumscription of the genus Alternaria which, among other things, allowed species which rarely formed chains of spores to be admitted to the genus.

On the basis of his cultural work and examination of the type and authentic specimens, he proposed a new combination, A. cucumerina, and relegated A. brassicae var. nigrescens to synonomy. The considerations which prompted this change and synonomy are not discussed in detail. Nattrass (41) isolated a fungus from watermelon which was determined to be A. cucumerina upon comparison with the type material. He furthar compared his isolate with a specimen of A. brassicae var. nigrescens as distributed in Briosi and Cavara's "Fungi Parassiti" and concurred with Elliot's proposal that the latter fungus was identical with A. cucumerina. Thus, in the opinion of 2 investigators, these names apply to 1 organism.

Table 1 presents a compilation of the conidial measurements made by various authors. Elliot's measurements were made from an isolate identified as A. brassicae var. nigrescens when he received it and, since he proposed it as a synonym, the measurements can seemingly be considered to refer to A. cucumerina. The writar has not examined the type specimens of either of the fungi in question and cannot, on this basis, express an opinion concerning the synonomy of the names. Numerous measurements have been made of conidia from many cucurbit isolates. The size range of the conidial body and beak is large, and the measurements presented in Table 1, taken from samples of unknown sizes, cannot be considered to entirely charactarize the organism. In the writer's opinion, the reported conidia dimensions and other morphological features ara valuable only as a general indication of the nature of the fungus.

Symptoms on the same host, as described by various authors, ara of limited value. Paglion (47) described muskmelon symptoms which ara very similar to those which have been reported most commonly by other investigators. Symptoms raported by Ellis and Everhart (16) from infected

muskmelon leaves were characteristic except that the diameters of the lesions were small. However, lesion size is perhaps the most variable muskmelon symptom. In view of the overlapping morphological features and the similarity of reported symptoms and host ranges, the writer agrees with Elliot in the synonomy of A. brassicae f. nigrescens Pegl. (A. brassicae var. nigrescens Pegl.) with A. cucumerina (E. & E.) J. A. Elliot and considers the latter as the valid name.

TABLE I

COMPARISON OF CONIDIA MEASUREMENTS IN MICRONS AS REPORTED BY VARIOUS AUTHORS

Name	Length	Width	Cross Septa	Citation
A. brassicae f. nigrescens	60- 85	14-20	6-8	Peglion (47)
A. brassicae f. nigrescens	100-160[a]	14-20	6-8	Peglion (48, 49)
A. brassicae f. nigrescens	100[a]	14-20	6-12	Saccardo (53)
A. brassicae f. nigrescens	100-130[a]	20-26	-	Nicolas (44)
A. brassicae f. nigrescens	23-110[b]	8-40[b]	-	Elliot (15)
A. brassicae f. nigrescens	54- 90	14-22	5-9	Neergaard (42)
M. cucumerinum	30- 75	15-25	3-8	Ell. & Ev. (16)

[a]Measurements include beaks.
[b]Data taken from graphs with modes about 38-75 x 17-20μ.

Neergaard (42) examined type specimens of A. brassicae var. nigrescens and concluded that the fungus was distinct from A. brassicae (Berk.) Sacc. and should be considered a species. He proposed the combination of A. nigrescens (Pegl.) Neerg. This combination, in view of the synonomy described above, is nomenclaturally superfluous, and it should be considered

as a synonym. Although A. brassicae f. nigrescens was described in 1893 and M. cucumerinum in 1895, no question of the priority of the former name is involved, since Article 60 of the International Code of Botanical Nomenclature (27) states that a name or epithet has no priority outside its own rank. When M. cucumerinum was described, its authors, in effect, raised form nigrescens to specific rank since the organisms involved were identical. Recommendation 60A2 of the Code (27) concerns the raising of infraspecific categories to specific rank and suggests that the new specific epithet be the same as the old infraspecific epithet unless otherwise prohibited. This procedure, however, is not mandatory. Elliot had no choice of names when he formed the combination A. cucumerina, but was obliged to use a derivation of the previous species name as prescribed by Article 55 of the Code. The correct name of the Alternaria leafspot pathogen, therefore, is Alternaria cucumerina (E. & E.) J. A. Elliot, Am. J. Botany 4: 439, 1917. The following invalid synonyms are recognized: (1) Macrosporium cucumerinum E. & E., Proc. Acad. Nat. Sci. Phila., p. 440, 1895; (2) Alternaria Brassicae f. nigrescens Pegl., Riv. patol. vegetale 1: 297, 1893 [also cited as A. brassicae var. nigrescens Pegl., Riv. Pat. Veg. 1: 296, 1893, according to Saccardo (53)]; (3) Alternaria nigrescens (Pegl.) Neerg., Danish Spp. Alternaria and Stemphylium, p. 232, 1945.

Elliot (15) did not redescribe A. cucumerina, and the only extant descriptions are those of Ellis and Everhart (16) and Peglion (47, 49). To clarify the circumscription of the taxon, the following description, which is based on Florida specimens, is proposed as an emendation of the present descriptions. All data are derived from structures taken from host material unless otherwise specified.

Foliage lesions are circular to irregular, barely depressed, at

times concentrically zoned, 1-15 mm in diameter or rarely larger, light brown to black, frequently confluent, becoming dry and membranous. Hyphae in culture are hyaline to olivaceous, branched, irregularly septate, 4-9μ in diameter, white to dark gray in mass. Conidiophores are maculicole, epiphyllous and hypophyllous, fasciculate to solitary, light to dark brown, geniculate or straight, 15-72 x 5-7μ, 1-6 septate with slightly enlarged bases. Conidia are mostly apical on conidiophores, light brown, ovoid to obclavate with few longitudinal septations when young, becoming dark brown, obclavate to fusiform-obclavate with 1-10 longitudinal septations when mature. The conidial body is smooth to papillate, 33.6-129.4 x 13.4-33.6μ, averaging 56.6-86.5 x 18.1-20.8μ from 680 measurements from 7 isolates. Mature conidia have a range of 5-13 transverse and 1-10 longitudinal septations, moderately constricted at the transverse septations. Beaks are straight, slender, short to long attenuate, quite variable in length, hyaline, occasionally with an enlarged basal cell which is contiguous with the conidial body, smooth, 2-6 septate, 20-336 x 1.3-5.0μ, averaging 106-121 x 2.2-2.4μ. In culture, conidia occasionally form in 2-spore chains.

Morphology

Mycelium and Conidiophores

Characteristics of the mycelium in culture vary greatly depending on the medium used, amount of light, temperature, humidity, and the genetic variability of the particular isolate. Various media were used initially in this study, and 18 per cent V-8 juice agar as described by Miller (38) was found to be favorable for growth and sporulation. This medium has been used almost exclusively and the mycelial characteristics described here were taken from growth on this medium.

Hyphal strands are 4-9μ in diameter, hyaline to olivaceous, somewhat submerged or closely appressed to the medium with sparse aerial growth. Hyphae branch abundantly and have numerous irregularly spaced septations.

Conidiophores in culture are brown, smooth, straight, and arise mainly from submerged mycelium. Frequently many conidiophores arise from a single ascending hyphal strand through formation of numerous terminal and lateral branches (Fig. 9). Aerial hyphae may give rise to a few lateral conidiophores which are much shorter and lighter in color. In nature conidiophores are maculicole, epiphyllous, and sparsely hypophyllous on most hosts. They project out at almost right angles to the lesion surface and each usually supports a single conidium at its apex. The dimensions of a sample of 36 conidiophores taken from several muskmelon lesions are 15-72 x 5-7μ with an average size of 37.6 x 6.6μ. They are smooth, straight or geniculate, fasciculate to solitary, brown, 1-6 septate with slightly enlarged basal cells. Conidia have not been observed to be borne laterally. Dark lateral hila can be seen which suggest that conidiophores, after having shed a conidium, continue to elongate and produce more conidia.

Conidia

The conidium is the only known spore form of the fungus. A conidium in culture develops at the apex of a conidiophore by an enlargement of the apical end, followed shortly by the formation of a septation below the enlarged apex. A deep constriction develops at the point of this septation and the young conidium thus is delimited. The conidium is at first ovoid, pale brownish yellow, and non-septate. Transverse septations develop rather rapidly and the conidium elongates and becomes somewhat

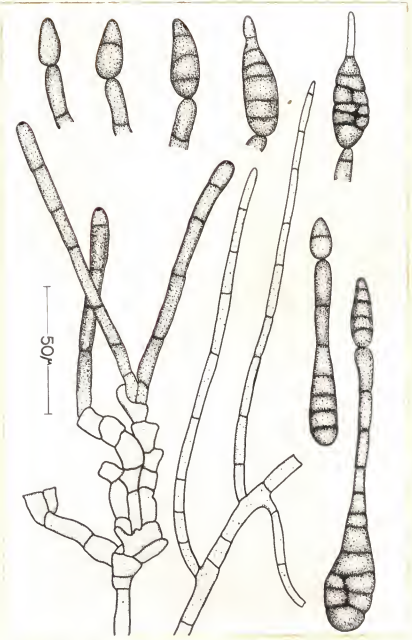

Fig. 9.--Camera lucida drawings of conidiophores and supporting mycelial processes at left, vegetative mycelium in center, and initiation of 2-conidia chains at right. Conidia at top represent a composite developmental series. All structures taken from cultures of the pathogen.

obclavate as the apical beak is initiated. Primary longitudinal septations and additional transverse septations are formed during this period of elongation of the conidial body and beak. Further development results in the formation of a few more longitudinal and transverse septations and considerable widening of the conidial body due to growth of individual cells. At maturity the cells are moderately constricted at the septations (Fig. 10). Conidia formation in culture occurs within 12 to 18 hours. Occasionally the beak remains hyphae-like, wider than usual and light brown in color. A second conidium then may be initiated at the distal end of the beak in a manner similar to that described for initiation from conidiophores. Thus a 2-spore chain is formed at times, the distal conidium being younger but not differing essentially in morphological features from the supporting conidium. Elliot (15) and Neergaard (42) have called attention to secondary development which occurs as the mature spore ages. Such additional development of conidia in culture or from natural media has been noted. The conidial body enlarges with a concomitant increase of transverse and longitudinal septations. Such conidia are dark brown and obclavate to broadly ovoid.

Conidia are dark olivaceous brown, commonly obclavate to fusiform-obclavate, straight or slightly curved, with regularly spaced transverse septations. The surface is smooth to papillate, the latter condition being commonly found. Beaks are flexuous, short and narrowly hyphae-like to long attenuate. They are smooth, usually hyaline, 2-10 septate with occasional enlarged basal cells. The length variability of beaks from culture or natural sources is greater than that of the conidial body and for this reason they have been excluded or treated separately in conidial mensurations. While this division is desirable, it leads to some confusion

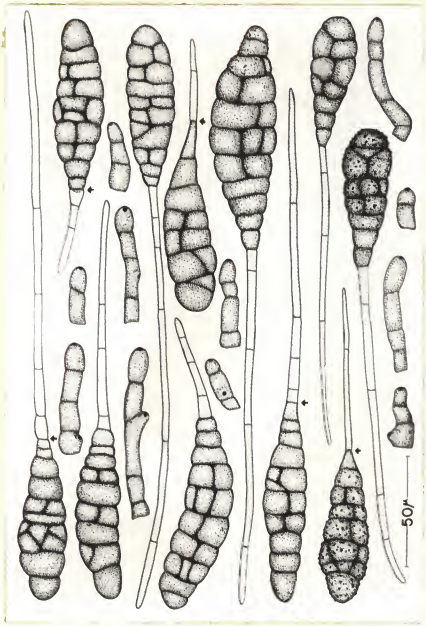

Fig. 10.--Conidiophores and conidia of A. cucumerina from host material drawn with the aid of camera lucida. The 2 conidia on the right show papillations which occur occasionally. Arrows indicate the points of beak-body junction. Conidiophores show the characteristically dark hila.

in the interpretation of reported measurements unless a carefully followed set of criteria are given which actually define the extent of the body. Neergaard (42) used the criteria of color, shape, and distance between septations in distinguishing between the conidial beak and body. Figure 9 shows examples of distinctions between beak and body in situations most frequently encountered. Color is the most valuable criterion as the beaks are usually uniformly hyaline and the point of union with the body can be readily seen. The conidial body tapers rather abruptly to the point of junction with the beak and beyond this point the beak diameter becomes only slightly smaller throughout its length. This point of junction is clearly defined in most cases. Examples are encountered in which a few terminal cells of the conidial body have approximately the same diameter as the base of the beak. These cells have been consistently taken to be part of the conidial body if their color was similar to that of the body. Occasionally, the cell wall of the apical body cell projects convexly into the lowest beak cell and this projection has been used in conjunction with other features mentioned to define the extent of the body. While the distinction between these 2 parts of the conidium may be artificial, it is interesting to note that when external force is applied, the conidium very often breaks at the point of junction as determined by the criteria mentioned.

The various measurements made of conidia from Florida isolates are given in Tables 2, 3, 4, and 5. Conidia from 9-to 12-day-old cultures grown on 18 per cent V-8 agar and from lesions resulting from natural or artificially induced infections were used. The required number of conidia in the samples were picked at random by scanning a microscope slide with the aid of a mechanical stage. Only conidia which contained at least one

TABLE 2

CONIDIAL BODY LENGTH IN MICRONS TAKEN FROM SAMPLES OF EIGHT ISOLATES FROM MUSKMELON (M), WATERMELON (W), AND SQUASH (S)

Isolate	Medium	Sample size	Mean length	s_x	s	CV
M-20	V-8 agar	50	54.4	1.5	10.4	19.2
M-20	V-8 agar	35	61.6	1.8	10.7	17.3
M-20	Muskmelon	35	86.5	2.9	17.0	19.6
W-34	V-8 agar	50	66.7	1.7	12.1	18.1
M-35	V-8 agar	50	66.0	2.0	14.2	21.4
M-35	Muskmelon	50	73.0	1.9	13.6	18.6
S-36	V-8 agar	50	49.8	1.1	7.7	15.5
S-36	V-8 agar	35	57.1	1.3	7.6	13.3
S-36	Squash	34	56.6	2.4	14.1	24.8
S-36	Watermelon	35	71.3	1.7	10.3	14.5
W-37	V-8 agar	50	61.1	1.6	11.2	18.3
W-37	V-8 agar	35	57.8	1.6	9.7	16.7
W-37	Watermelon	35	76.1	2.6	15.3	20.0
M-38	V-8 agar	50	64.9	2.1	14.8	22.9
M-38	Muskmelon	50	79.9	1.1	8.1	10.0
M-40	Muskmelon	50	69.6	1.7	12.0	17.2
M-41	V-8 agar	50	56.1	1.6	11.1	19.8
M-41	Muskmelon	50	66.4	1.4	10.0	15.1

TABLE 3

CONIDIAL BODY WIDTH IN MICRONS TAKEN FROM SAMPLES OF EIGHT ISOLATES FROM MUSKMELON (M), WATERMELON (W), AND SQUASH (S)

Isolate	Medium	Sample size	Mean width	s_x	s	CV
M-20	V-8 agar	50	17.5	0.3	2.2	12.7
M-20	V-8 agar	35	19.5	0.4	2.3	11.6
M-20	Muskmelon	35	18.8	0.4	2.4	12.6
W-34	V-8 agar	50	18.0	0.4	2.5	13.7
M-35	V-8 agar	50	24.8	0.6	4.3	17.1
M-35	Muskmelon	50	20.0	0.4	2.5	12.7
S-36	V-8 agar	50	20.9	0.4	2.6	12.4
S-36	V-8 agar	35	22.2	0.5	2.8	12.5
S-36	Squash	34	18.1	0.8	4.4	22.8
S-36	Watermelon	35	18.1	0.3	1.7	9.6
W-37	V-8 agar	50	21.6	0.3	2.4	11.3
W-37	V-8 agar	35	20.5	0.5	2.9	14.3
W-37	Watermelon	35	21.0	0.6	3.3	15.6
M-38	V-8 agar	50	19.4	0.5	3.4	17.6
M-38	Muskmelon	50	18.5	0.4	2.6	14.1
M-40	Muskmelon	50	20.8	0.7	4.9	23.5
M-41	V-8 agar	50	20.8	0.5	3.4	16.4
M-41	Muskmelon	50	18.1	0.4	2.5	13.7

TABLE 4

BEAK DIMENSIONS IN MICRONS TAKEN FROM SAMPLES OF THREE ISOLATES OF MUSKMELON (M), WATERMELON (W), AND SQUASH (S)

Isolate	Medium	Mean length[a]	$s_{\bar{x}}$	s	CV	Mean width
M-20	V-8 agar	217.4	10.1	59.9	27.5	2.2
M-20	Muskmelon	121.4	9.6	56.9	46.9	2.2
S-36	V-8 agar	142.8	12.6	74.4	54.2	2.7
S-36	Watermelon	106.0	7.7	45.3	42.7	2.2
W-37	V-8 agar	156.5	6.2	57.7	36.8	2.7
W-37	Watermelon	107.1	7.4	44.0	41.2	2.4

[a]Sample size was 35 in each case.

TABLE 5

NUMBERS OF SEPTATIONS AND CELLS OF THE CONIDIAL BODY TAKEN FROM SAMPLES OF THREE ISOLATES OF MUSKMELON (M), WATERMELON (W), AND SQUASH (S)

Isolate	Medium	Mean number of septations[a]		Number of cells[a]	
		Transverse	Longitudinal	Mean	Range
M-20	V-8 agar	6.9	5.5	13.3	8-18
M-20	Muskmelon	9.1	4.7	14.7	7-22
S-36	V-8 agar	6.5	5.5	12.7	8-18
S-36	Watermelon	6.8	3.0	10.7	8-15
W-37	V-8 agar	6.0	5.5	12.2	8-18
W-37	Watermelon	7.5	3.8	12.5	9-20

[a]Sample size was 35 in each case.

longitudinal septation were included since non-muriform spores were regarded as immature. Septations that were visible in the uppermost plane of focus were counted. Septations which were continuous in approximately straight lines, even though intersecting several other septations, were counted only once. The greatest overall diameter of the conidial body and the diameter of the beak at its mid point were recorded.

Several descriptive values were calculated for each sample. These values are necessary for an accurate appraisal of the variability of the morphological features under consideration. Standard deviation (s) is an indication of the variation which occurs in any particular sample, indicating by its relative size whether most sample values ranged closely about the mean or were widely scattered. Standard deviation was calculated by the formula

$$\sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

where X represents the individual values of the sample and n is the sample size. To compare the variability of different samples which are not equal in size or to compare the variability of 2 different characteristics such as length and width, a relative measurement must be computed. The coefficient of variation (CV), s/mean x 100, is a better comparative measure of dispersion because it is a relative value. Since the above descriptive terms depend largely on the value of the mean, an indication of the reliability of the mean can be obtained by calculating the standard error of the mean ($s_{\bar{x}}$) as the square root of s^2/n. When the standard error is added to and subtracted from its mean, a range of values is obtained. It can be said with approximately 68 per cent confidence that the parameter, μ, lies within this range.

Mean conidial body length and width as shown in Tables 2 and 3 were not excessively variable among isolates. Among samples of the same isolate, the mean length of conidia from a natural medium generally was greater than those from V-8 agar. One exception, a sample of S-36 from Hubbard squash, was found. The mean width frequently was less on natural medium. The standard deviations agreed in magnitude with those obtained by Neergaard (42) for many of the species of Alternaria which he studied. The standard deviations of the samples indicated a wide range of dimensions for the muriform conidia of this species. Judging from any particle sample, the range of dimensions which could be expected in 99.7 per cent of the population would be represented by the mean plus or minus 3 standard deviations. The size of the sample would patently affect the value of such a calculation, as larger samples generally result in greater reliability of the derived quantities such as mean and standard deviation. The length and width range of the natural-medium sample of isolate M-35, which does not represent an extreme, can be used as an example. Calculating from the sample characteristics shown in Tables 2 and 3, 99.7 per cent of the conidia in the M-35 population would be within the range of 32.2-113.8 x 12.5-27.5μ in size. The reliability of the means for length and width appears to be relatively good, since the standard errors of the means are small compared with the magnitudes of the means. The coefficients of variation for each sample permit a direct comparison of the variability of the sample dimensions presented in Tables 2, 3, and 4. Such comparisons show that body width is usually less variable than body length. Beak length is from 2-5 times more variable than either body length or width, a fact that reaffirms the desirability of treating the 2 structures separately. These data do not indicate that the conidial body from culture is more or less

variable than that from natural media. However, beak length appears to be more variable in culture than from natural media.

Physiology

The fungus grows well on 2 per cent potato-dextrose agar (PDA), 18 per cent V-8 juice, Czapek's agar, and a nutrient agar to be described under the discussion of temperature relations. A sparse growth is obtained on a minimal liquid medium described by Newton (43) which contains 0.2 g of both potassium biphosphate and potassium nitrate plus 20 g of glucose per liter of water solution. Several isolates have exhibited chromogenesis ranging from yellow on PDA to red on V-8 agar. The characteristic growth habit described under morphology is quite variable, and when grown on PDA the mycelium becomes aerial and cottony. On very acid or very alkaline media, hyphal strands tend to become moniliform, and growth is very slow. While the medium exerts a great influence on the growth aspect, the genetic differences which occur among different isolates and among single-conidial isolations from the same sources have resulted in great variations in growth form. In the present study the isolates which have failed to sporulate or have sporulated poorly have not been considered. Manipulation of pH, nutrients, and temperature as well as procedures such as cutting, scarifying, or drying the cultures have been ineffective in increasing spore formation. Solar or ultra violet irradiation also have been ineffective in this respect.

Germination of Conidia

Conidia germinate rapidly under favorable conditions in a manner very similar to that described by Weber (64). To investigate the relation of temperature to germination, an experiment was devised using conidia of isolates M-20, S-36, and W-37. These isolates were grown on V-8 agar for

10 days at 28°C. Conidia were harvested by flooding each culture with distilled water to which 2 drops of "Tween 80" had been added. The surface of each culture was brushed gently to loosen the conidia and the resulting suspension was strained to remove fragments of mycelium. The spore suspensions thus obtained were about pH 6.0±0.3. Petri dishes were used as moisture chambers and filter papers moistened with distilled water were placed in the lower halves to maintain constant high humidity. Prior to placing the slides within them, petri dishes were placed in constant temperature chambers at 4°C intervals from 4° to 44°C for 1 hour to bring them to their respective temperatures. Clean glass microscope slides were sprayed with the spore suspensions and placed on supporting glass rods just above the filter paper. Two slides of each isolate were used in each petri dish and the three isolate series were replaced in their respective boxes. At the end of 4 hours, all slides were sprayed gently with 50 per cent lacto-phenol and stored temporarily at 8°C until they were examined. Each slide was inspected microscopically by placing a cover slip over the spore suspension and scanning the slide with the aid of a mechanical stage. Only cells of the conidial body were included in the measurements. The number of germinated cells and the lengths of the germination hyphae were recorded for each isolate-temperature combination. A minimum of 35 measurements were made of germination hyphae which were rarely branched after 4 hours. At least 25 conidia of each treatment combination were inspected to determine the number of germinated cells. All samples were taken at random. The mean values of these observations are presented in Figure 11. No germination occurred at 4°, 8°, or 44°C, and the optimum range for conidia germination was between 20° and 28°C. The isolates differed somewhat in their ability to germinate at 12° and

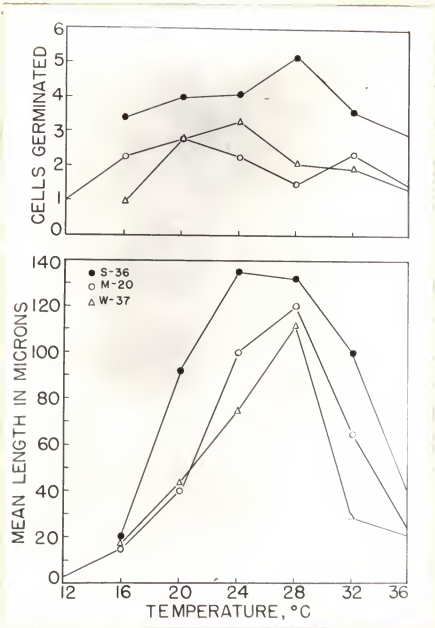

Fig. 11.--Mean length of germination hyphae and mean number of cells germinated at 8 temperatures.

36°C and in average numbers of germinated cells at these temperatures.

Relation of Temperature and pH to Growth of the Fungus

Brisley (7) reported that the fungus grew slowly in an alkaline or neutral medium and that it responded to a slightly acid medium by growing rapidly. The hydrogen-ion concentrations used in his studies were not specified. He reported that the cardinal temperatures for growth of the fungus were 8°, 27°, and 45°C, with good to excellent growth occurring within a range of 21° to 27°C. His data were derived from growth of the fungus on solid media.

A preliminary series of V-8 agar cultures was incubated at 4°C increments from 4° to 44°C. The results of this preliminary experiment, based only on measurements of radial growth, are similar to those of Brisley; however, growth did not occur above 36°C.

The relation of the growth of the fungus on a synthetic medium to temperature and pH was determined primarily in a series of 2 experiments. The medium was devised to evoke rapid mycelial growth and contained the following amounts of reagent and C.P. grade chemicals:

Ammonium tartrate	2.0 g
Sodium nitrate	2.0 g
Potassium biphosphate	2.0 g
Magnesium sulfate	0.5 g
Calcium chloride	0.1 g
Glucose (anhydrous)	19.7 g
Minor element solution	1.0 ml
Ferrous sulfate solution	1.0 ml
Vitamin solution	10.0 ml
Distilled water	to make 1 liter

Each ml of the minor element solution was composed of 0.01 mg of boron as boric acid, 0.1 mg of copper as copper sulfate, 0.02 mg of molybdenum as ammonium molybdate, and 2.0 mg of zinc as zinc sulfate. The ferrous sulfate solution contained 0.2 mg of iron per ml. The vitamin solution was added to promote rapid growth and each ml was composed of 0.025 µg of

biotin, 0.1 mg of thiamine hydrochloride, 0.05 mg of riboflavin, 0.05 mg of pyridoxine hydrochloride, 0.2 mg of pantothenic acid, and 0.05 mg of choline chloride.

All components of the medium were prepared as stock solutions in quantities sufficient to supply the series of experiments which employed this medium. The stock solutions were stored at 4° and 12°C, and the medium was prepared by diluting required amounts with distilled water.

Relation of temperature to growth with constant initial pH.--An experiment was performed to explore the responses of 3 isolates to various temperatures using the same initial pH value. Thoroughly washed 500 ml erlenmeyer flasks were rinsed with distilled water, oven dried, and plugged with uniformly fashioned cotton plugs. After the addition of 100 ml of medium to each, the flasks were autoclaved at 15 pounds pressure for 15 minutes. The final pH of the medium was 7.5±0.3, as determined by a 5 per cent sample. The medium in each flask was adjusted aseptically to pH 6.2±0.3 with 0.5 N HCl. An additional 5 per cent sample of the adjusted medium was made and the results were used as the basis of the pH error estimate.

Isolates M-20, S-36, and W-37 were grown initially in separate petri dishes on a medium prepared by adding 20 g of refined agar to 1 liter of the synthetic medium previously described. Uniform-size discs, 3 mm in diameter, of the medium and fungus mycelium were cut from the perimeters of 6-day-old cultures and placed in randomly chosen flasks. The temperatures that were used ranged from 8° to 36°C in 4°C increments. Three replications were used for each isolate-temperature combination. The prepared flasks were assigned at random to the constant temperature chambers and were not disturbed for 9 days. Growth of the isolates at various

temperatures was determined by weighing the mycelial mats after drying at 36°C for 4 days.

A statistical analysis of the factorial experiment was based on a design which considered the flask plus medium as the experimental unit to which the treatment combinations of temperatures and isolates were assigned at random. An analysis of variance of the results obtained is given in Table 6.

TABLE 6

ANALYSIS OF VARIANCE IN GROWTH OF THREE ISOLATES AT EIGHT TEMPERATURES

Sources of variation	d.f.	M.S.
Isolates	2	349100.5**[a]
Temperatures	7	576161.2**
Interaction (I x T)	14	48817.0**
Error	48	3665.4
Total	71	

[a]Double asterisks denote significance at least at the 1 per cent level.

The analysis of variance indicated that the responses to temperature were different and also that the isolates differed in growth responses at these temperatures. The interaction, while significant, was small compared with the main effects and suggested that relative responses of isolates to a given temperature may differ slightly.

Since the analysis of variance does not indicate which temperatures were different from one another, the multiple range test of Duncan (13) was used to test the differences which occurred among mean growth responses

at the 8 temperatures. Since the magnitude of the interaction mean square was very small compared with those of the main effects of isolates and temperatures, testing the overall affect of temperatures and isolates was justified. Using $S_m = \sqrt{\frac{3665.4}{9}}$ as the standard error for a temperature mean and Duncan's tables (15) of significant studentized ranges for the 5 per cent level of significance, the following shortest significant ranges were calculated:

p:	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Rp:	57.7	60.8	62.6	64.0	65.0	66.0	66.6

Mean growth at each temperature, expressed as mg dry weight of the mycelium, was derived from all replications of each isolate grown at a given temperature. The mean values were ranked in order of their increasing magnitudes and tested against Rp above.

Temperatures:	36	12	8	16	32	20	24	28
Mean growth:	31.1	69.7	74.6	209.6	325.1	330.1	612.2	672.7

All means underlined by the same line are not significantly different and means not underlined by the same line are significantly different.

The results of the test showed that mean growth of isolates at 24° and 28°C was not different but that mean growth at these temperatures was significantly greater than mean growth at any other temperatura tested. The growth responses of each individual isolate to the temperature series was tested in a manner similar to that described above but using the appropriate values for S_m and Rp. These tests indicated that the relative order of the temperatures and the relative significance of the means were essentially the same for each isolate and were closely comparable to that presented above. Duncan's test also was applied to the isolates since the isolate mean square indicated significant differences. All isolates differed

from one another when tested at the 5 per cent level.

A least squares analysis of the experimental data from each isolate was performed. The cubic fit was better in each case than those obtained by using linear, quadratic, or fourth degree expressions. The area of maximum response can be defined more clearly by reference to Figure 12. Maximum response occurred between 24° and 28°C, or within a range which included temperatures slightly below and above 24° and 28°C respectively.

Relation of pH growth of the fungus at various temperatures.--In view of the results of the previous experiment, the 4 temperatures that evoked most response were chosen to form temperature-pH treatment combinations. Initial hydrogen-ion concentrations of 4.0, 5.0, 6.2, and 7.7 were chosen to cover a range of values within which the fungus might be expected to grow. To eliminate the possibilities of interactions involving isolates, only 1 isolate, W-37, was used in the experiment.

The preparation of flasks and medium was similar to that described for the previous experiment. The final pH of the autoclaved medium was 7.0 ± 0.3, as determined by a 5 per cent sample. Four groups of 15 flasks each were chosen at random and the medium adjusted aseptically to the desired pH values. Three of the flasks, containing medium adjusted to each of the 4 pH values, were selected at random and used as the bases for pH error estimates. The hydrogen-ion concentrations were determined to be 4.0 ± 0.2, 5.0 ± 0.1, 6.2 ± 0.2, and 7.7 ± 0.2. The medium in the remaining 12 flasks of each pH group was inoculated with uniform-size discs, 3 mm in diameter, of agar plus mycelium cut from the perimeters of 6-day-old cultures of isolate W-37. Three replications of each pH group were assigned to each of the 4 temperatures and the resulting groups of 12 flasks were

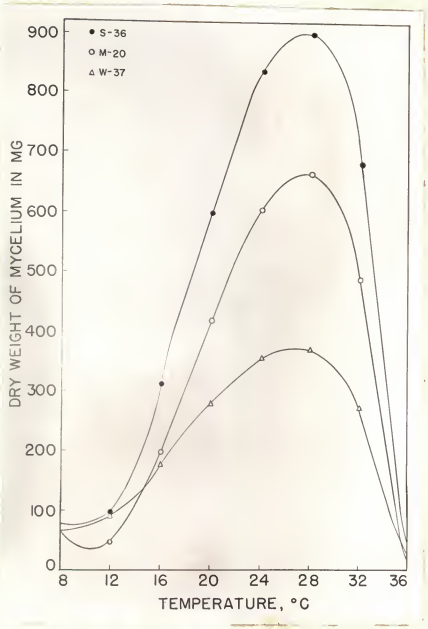

Fig. 12.--Derived response curves showing relationship of growth of 3 isolates to temperature at pH 6.2

randomly arranged in the 20°, 24°, 28°, and 32°C constant-temperature chambers. The flasks were not disturbed for 11 days. Growth of the isolate under each of the 16 temperature-pH conditions was determined by weighing the mycelial fragments after drying at 36°C for 4 days. The filtrates from the 3 replications of each treatment combination were combined and the pH of each combination was recorded (Table 7).

TABLE 7

FINAL pH VALUES AT INDICATED TEMPERATURES FROM COMBINED FILTRATES OF THREE REPLICATIONS OF EACH TREATMENT COMBINATION

Treatment		Final pH
Temperature	pH	
20	4.0	4.4
20	5.0	4.6
20	6.2	5.9
20	7.7	7.2
24	4.0	4.3
24	5.0	4.6
24	6.2	6.1
24	7.7	7.1
28	4.0	4.2
28	5.0	4.2
28	6.2	5.9
28	7.7	7.1
32	4.0	4.4
32	5.0	5.3
32	6.2	5.8
32	7.7	6.8

At pH 7.7, mycelial growth at all temperatures was submerged below the surface of the medium while at pH 5.0 and pH 6.2, floating mycelial mats and emergent masses of hyphal strands were formed. Very little growth occurred at pH 4.0. The final pH values of the combined filtrates in many instances were quite close to the initial values.

The results of the experiment were subjected to an analysis of variance which is shown in Table 8.

TABLE 8

ANALYSIS OF VARIANCE IN GROWTH OF ONE ISOLATE AT SIXTEEN TEMPERATURE-pH COMBINATIONS

Sources of variation	d.f.	M.S.
Temperatures	3	9212.63**[a]
pH	3	20192.30**
Interaction (T x pH)	9	2941.03
Error	32	1605.56
Total	47	

[a]Double asterisks denote significance at the 1 per cent level.

Differences were found in the mean growth responses to the 4 temperatures and also to the 4 pH values. The interaction between the 2 factors was not significant at this level or at the 5 per cent level of significance. Duncan's multiple range test (13) was employed to specify the significantly different means. Using $S_m = \sqrt{\frac{1605.56}{9}}$ as the standard error for a temperature or pH mean and Duncan's tables of significant studentized ranges for the 5 per cent level of significance, the following shortest significant ranges were calculated:

p:	(2)	(3)	(4)
Rp:	33.43	35.17	36.09

The mean growth responses in mg at each of the 4 temperatures were ranked according to their increasing magnitudes and tested against the Rp values above:

Temperature:	24	32	20	28
Mean Growth:	60.08	61.75	63.08	117.00

A similar ranking of the mean growth responses in mg at each of the 4 pH values was made and tested against the Rp values above:

pH:	4.0	7.7	6.2	5.0
Mean growth:	34.59	53.75	85.08	128.50

All means underlined by the same line are not significantly different and values not underlined by the same line are significantly different.

The test of the ranked means resulting from growth at 4 temperatures indicates that growth was significantly greater at 28°C and that growth at all other temperatures was not different. These results, involving growth over a range of pH values, are similar to the results obtained for the same isolate in the first experiment which employed varying temperatures and a constant initial pH of 6.2.

Mean growth in the medium having an initial pH of 5.0 was greater than growth at any other pH. The responses at pH 7.7 and pH 6.2, and at pH 7.7 and pH 4.0 were not significantly different from each other.

Pathogenicity

The pathogen has been isolated consistently from cucurbit leaf lesions, and the isolates thus obtained have proven to be pathogenic to a variety of cucurbits in greenhouse tests. Reisolation from these artificially inoculated plants has yielded the same fungus without exception.

The relation between age of the host tissue and degree of infection by the pathogen has not been discussed in the literature concerning the disease. Watermelon plants frequently are found with scattered lesions on the older leaves suggesting that these leaves are more susceptible because of their greater age. The majority of the informative reports of the pathogen on various cucurbits mention the disease occurring at or after flowering, which implies a certain physiological maturity of the hosts.

An experiment was initiated to discover the relationship between host age and susceptibility to the pathogen. Black Diamond watermelon, Rio Gold cantaloupe, Yellow Summer Crookneck squash, and Marketer cucumber were used as hosts. Seeds of the various hosts were planted in fumigated soil in boxes 11 x 13 x 31 inches. Two complete replications of the 4 hosts were planted every 10 days until 4 such groups were obtained. Each replication consisted of about 40 plants of each host. Host vigor was maintained by frequent applications of a 6-6-6 commercial fertilizer. The boxes were randomized on the greenhouse bench and 1 replication of each age group was used as a control. Inoculum consisted of conidia from pooled cultures of all isolates, grown on V-8 agar for 14 days at 28°C. Conidia were loosened from the mycelium by brushing gently with a camel's hair brush in the presence of water. The inoculum was placed in a 3-gallon, pressure-type sprayer and sprayed on the plants. The boxes containing the control plants were covered with plastic while the remaining plants were being inoculated.

Inoculated plants of all ages became infected to some extent. The greatest number of lesions developed on those hosts that were 40 days old when inoculated. Leaf drop and subsequent death of plants was pronounced in the case of squash. Moderate leaf infection occurred in the 30-day-old

group on all hosts except cucumber which was only slightly infected. Lesions enlarged slowly and plants outgrew the infection primarily, it is believed, because of conditions unfavorable to reinfection. Infections occurred on all hosts of the 20-day-old group, but lesions did not develop to more than 1-3 mm in diameter. This group shed the infected leaves in the normal course of growth and development of the plants. The cotyledons of each host were susceptible to attack as was shown by the results from the 10-day-old group. No true-leaf infection was observed. Squash cotyledons were infected, but no distinct lesions formed. These organs became chlorotic and died within 10 days. Cucumber cotyledons became slightly flecked but did not develop chlorosis. The infection of watermelon and muskmelon cotyledons was accompanied by the formation of large, rapidly expanding, circular brown lesions. Such lesions quickly coalesced and the entire cotyledon eventually became necrotic. Invasion of the hypocotyl tissue did not occur. These hosts were susceptible to the pathogen at all stages of growth, but infection of young plants, unless accompanied by conditions very favorable for the growth and spread of the pathogen, did not result in severe foliage damage.

Alternaria leafspot frequently was associated with gummy stem blight or anthracnose of watermelon and with downy mildew of muskmelons. Alternaria conidia commonly have been observed forming on the adaxial surface of a somewhat angular, necrotic lesion on muskmelon leaves while sporangiophores of the downy mildew pathogen occurred on the abaxial surfaces of the same lesion. These observations suggest that the Alternaria leafspot pathogen may have become established in necrotic tissue resulting from infection by other pathogens. However, isolates of the Alternaria leafspot pathogen which have been derived from these situations have been

found to be pathogenic in the absence of other pathogens, wounds, or necrotic tissue.

A second species of Alternaria frequently was found associated with the above mentioned diseases of watermelon and muskmelon. It has been tentatively identified as A. tenuis auct. sensu str. and was found forming catenulations of conidia on the surface of necrotic tissue. Greenhouse inoculations with conidia of this fungus have never resulted in the infection of any cucurbit, and it is believed to be entirely saprophytic with respect to cucurbit foliage.

Isolation and Inoculation

The pathogen has been isolated readily from numerous cucurbit leaf lesions. The lesions were examined under low magnification with a stereoscopic binocular microscope, and when abundant conidia were found they were brushed off onto a water agar surface for further examination. Isolated conidia were picked from the surface of the agar with a sterile needle and placed on V-8 agar in tubes. This method resulted in a very high percentage of uncontaminated single-conidial isolations. Many isolations were made in this way from each collection of diseased material. All isolations from a given collection were numbered identically and formed the basis of the particular isolate. Isolations from all sources were cultured on V-8 agar in petri dishes. The majority of all isolations produced spores in culture without special treatment. Nine pathogenic isolates were obtained from various cucurbit hosts, and 6 of these were used in experiments to determine the host range of the fungus. Of the 6 isolates used, 3 were derived from muskmelon, 2 from watermelon, and 1 from squash.

Inoculations were performed in the greenhouse, and the materials and some of the methods used were common to all experiments. Plants were

maintained in a thrifty condition by application of a 6-6-6 commercial fertilizer, and insects and powdery mildew were controlled by semi-monthly applications of a mixture of lindane and karathane. Plants were grown in 8, 10, and 12 inch clay pots filled with fumigated soil. Inocula were prepared by suspending the conidia from 8-10 petri dish cultures in 2 1/2 liters of water which contained 3-5 drops of "Tween 80" as a detergent. The numbers of conidia thus obtained were from 800 to 1600 per ml as determined by several small samples of isolate suspensions. The suspensions of conidia were applied with DeVilbiss atomizers or with a 3-gallon pressure-type sprayer which was thoroughly flushed with water before application of each isolate. The relative humidity was maintained above 95 per cent for the first 12 hours following inoculation and at 60-95 per cent for the subsequent 4 days. Greenhouse temperatures fluctuated between 18.3° and 32.2°C for the entire period of the experiments. Three separate experiments were performed to determine the host range. The objective of each experiment was to discover which hosts were susceptible to the pathogen. No critical attempts were made to determine any possible difference in the virulence of the isolates employed.

Two common native plants, Melothria pendula L. (Cucurbitaceae) and Lepidium virginicum L. (Cruciferae), and 2 cultivated plants, Marglobe tomato and Marion Market cabbage, were used in the first experiment. The native species were collected from fields and woodlands around Gainesville, Florida. Fifteen pots of each type of plant to be tested were prepared and arranged at random on a greenhouse bench. Two replications of each type of plant were subjected to the same isolate treatment and 3 replications of each type of plant served as untreated controls. All plants were growing vigorously at the time of inoculation.

The control plants showed no evidence of infection or saprophytic growth 15 days after inoculation. Inoculated tomato plants did not become infected. Definite grayish leaf flecks developed on cabbage and L. virginicum although these flecks did not enlarge and no conidia could be observed on the surfaces of the flecks. At the time of inoculation, plants of L. virginicum had produced many branched inflorescences which bore numerous silicles, some of which were dry and mature. Two weeks after inoculation, all inflorescences were examined and many were found to be sparsely covered with conidiophores and conidia of the pathogen. The conidiophores had developed from the surfaces of silicles which were dry and mature. Immature green silicles were not observed to be affected. To verify the identity of the conidia found in this situation, isolations were made of the conidia taken from the surface of a silicle which bore many conidiophores. A spore suspension was prepared using the cultures of the isolate and atomized on muskmelon seedlings growing in flats. The seedlings inoculated in this manner developed symptoms of the disease within 3 days while adjacent untreated seedlings remained healthy. These observations indicated that the pathogen was able to become established in the dead tissue of the mature silicles and produce conidia for the possible reinfection of the host. Small yellow lesions developed on the leaves of Melothria pendula. Conidia were not produced on the surfaces of the lesions, but the pathogen was isolated from the diseased leaf tissue.

The second and third experiments were performed in a similar manner and the following comments apply to both of them. Plants to be tested were arranged in groups, each of which contained one planting of each type. Nineteen such groups were equally spaced on greenhouse benches. Three groups were selected at random for each of the 6 inoculation treatments

and 1 group served as the uninoculated control. Qualitative differences in isolate virulence were not observed and the results obtained from using all isolates are combined in Table 9. These results confirm most reports that have been made concerning the host range of the pathogen except for Brisley's report (7) which lists tomato and potato.

TABLE 9

RESULTS OF INOCULATION EXPERIMENTS IN THE GREENHOUSE GIVING MEAN PERCENTAGE OF INFECTION OBTAINED WITH SIX ISOLATES

Plant	Horticultural variety	Number inoculated	Mean percentage infected
Brassica rapa L.	Purple Top	70	0
Citrullus vulgaris Sch.	Black Diamond	50	34
Citrullus vulgaris Sch.	Citron	18	6
Cucumis anguria L.	-	68	28
Cucumis melo var. cantalupensis Naud.	Rio Gold	82	89
Cucumis melo var. inodorus Naud.	Honey Dew	65	86
Cucumis sativus L.	Marketer	65	90
Cucumis sativus L.	National Pickling	73	84
Cucurbita maxima Duche.	Boston Marrow	29	70
Cucurbita maxima Duche.	Hubbard	15	100
Cucurbita maxima var. turbaniformis Alef.	Buttercup	36	77
Cucurbita moschata Duche.	Butternut	70	95
Cucurbita pepo var. melopepo Alef.	Coccozelle Bush	37	68
Cucurbita pepo var. melopepo Alef.	Yellow Summer Crookneck	58	92
Cucurbita pepo var. ovifera Alef.	mixed varieties	39	80
Daucus carota var. sativa DC.	Danver's Half-Long	98	0
Momordica balsamina L.	-	60	52
Momordica charantia L.	-	20	80
Solanum tuberosum L.	Red Bliss	32	0

HOST-PARASITE RELATIONS

Seasonal Development of the Disease

Alternaria leafspot of cucurbits has been observed in central Florida during the frost-free months from March through November. Infection may occur before April in the counties in the southern parts of the state and is commonly seen as well established infections in the northern-central portion of the state by May or June. Severe outbreaks of the disease in Florida have been noted to occur only during the warmer months of May through September when there is moderate to heavy rainfall. In such instances, the disease usually is found in a limited area such as a few fields within an area of 5-10 square miles. Severe defoliation of susceptible cucurbits occurs within 4-6 weeks if control measures are not applied. Scattered light infection of watermelon is generally widespread in central Florida from April through July. Less than 5 per cent of the leaves usually become infected, and these leaves have only 1-10 lesions per leaf. This degree of infection is of little importance except as a source of inoculum for more severe infections. Alternaria leafspot has been observed on muskmelons during September, October, and November in fields where August plantings were made.

Natural infection of cucurbit seedlings has never been observed in the field. Infected cucurbits have been at least 30 days old in all cases, and usually mature enough to produce flowers.

Longevity of the Fungus

Several reported experiments have been performed to determine the longevity of conidia and mycelium *in vitro*. The results of these experiments have been used as evidence to support various conclusions concerning

the longevity of the fungus in field soil and plant debris. Brisley (7) studied the longevity of conidia and mycelium by taking samples of conidia and mycelium from a dried culture at progressive time intervals, suspending the samples in a hanging drop of prune agar, and recording the percentage of germination or renewed growth which occurred after 24 hours. The temperature at which the culture was stored was not specified. Brisley found that conidia became almost totally non-viable after 70 days while the mycelium was able to resume growth after 9 months storage. In view of these results, he concluded that the mycelium living in the dead host was responsible for carrying the fungus over winter, and that conidia did not contribute to this phase of the disease cycle. This view was in opposition to that of Blinn (5). Van Haltern (61) was able to recover viable conidia which had survived in flasks of steamed soil that were exposed during the winter.

An experiment was designed to investigate the effect of various constant temperatures on the longevity of conidia. Isolates S-36 and W-37 were grown in petri dishes on V-8 agar medium at 28°C for 20 days after which they were dried at 32°C for 10 days. Nine dry cultures of each isolate were obtained in this way. Prior to storage the viability of the conidia of the 2 isolates was determined by taking samples from 1 culture of each isolate series. Each sample was obtained by brushing the surface of the culture with a small camel's hair brush and then letting the conidia fall onto the surface of water agar in a petri dish. The samples were then placed at 28°C for 10 hours. The percentages of viable conidia were obtained by examining the samples with a stereoscopic binocular microscope and counting the germinated conidia occurring in a random inspection of 200 conidia. The percentage of germination in each sample

was taken to represent the condition of the remaining unsampled cultures. One culture of each isolate was assigned to 1 of 8 temperature conditions. Seven cultures of each isolate were stored in a dry condition. The conidia of 1 culture of each isolate were suspended in distilled water, and the suspension was divided into 4 equal portions, placed in closed vials, and stored at -8°C. When a sample of this group was desired, 1 vial was thawed and all conidia used and subsequently discarded.

Samples of conidia from each isolate-temperature combination were taken at various time intervals and treated in the same manner as described for the initial samples. The results of the experiment are presented in Table 10. The results indicate that viability of conidia was relatively unimpaired at some temperatures during the entire experiment while it progressively decreased at 42°, 24°, and -8°C. The reasons for the low viability of the W-37 isolate at 28°C were not discovered. The data, when plotted, gave a bimodal distribution curve for each isolate, reaching a maximum at 8°C and again at 28° or 36°C. In general, these data were in disagreement with the findings of Brisley (7), although the methods used were similar.

In vitro studies of the longevity of conidia, while furnishing valuable information concerning the durability of conidia, do not reflect many field conditions which tend to reduce the longevity of conidia. In addition to the longevity tests performed in the laboratory, 10 dry cultures of isolate W-37 were buried in a garden plot to investigate some of the effects of field environment on protected conidia. Petri dishes containing the dry cultures were wrapped with aluminum foil and placed at a depth of 6 inches. The cultures were unearthed and examined after 8 1/2 months. The absence of conidia and the paucity of mycelium in 6 of the 10 cultures

apparently was due to the attacks of insects which were found within the petri dishes. The remaining 4 cultures contained abundant conidia, many of which had germinated during storage in the soil. Ungerminated conidia were sampled in the same manner as that used in the previous experiment. The viability of conidia in a sample of 400 was less than 1 per cent, indicating that the soil environment was less favorable for the existence of these protected conidia.

TABLE 10

RELATION OF THE PERCENTAGE OF GERMINATING CONIDIA TO LENGTH OF STORAGE OF TWO ISOLATES AT EIGHT TEMPERATURES

Temperature	Isolate S-36[a]			Isolate W-37		
	75[b]	200	255	75	200	255
-8 (suspension)	31	11	10	93	13	3
-8	100	0	2	100	90	4
8	99	94	93	99	99	99
16	87	-	-	97	-	-
24	91	73	24	92	51	14
28	97	98	96	98	78	44
36	90	83	88	98	94	95
44	91	74	45	93	79	18

[a]Initial percentage viability was 98 for S-36 and 99 for W-37.
[b]Days after initial sampling and storage.

Sources of Inoculum

Attempts to artificially produce the disease, using suspensions of mycelial fragments and carefully controlled temperature and relative humidity, have been unsuccessful. In view of these results and the absence of reported information concerning the manner in which initial infection

has occurred, it is believed that conidia must be present in the field before infection can occur. The mycelium living in dead vines was thought by Brisley (7) to be responsible for the supply of spring inoculum. Lindford (32) reported that the disease was severe in fields which previously had been planted with muskmelon. Martin (33) believed that the fungus lived from one season to another in the dead vines since the disease seemed most serious in fields which had been planted repeatedly with melons. No valuable contribution to the understanding of the problem was made until Van Haltern (61) presented experimental evidence which showed that the disease developed rapidly on muskmelons planted on land which had been used previously for muskmelons, but did not develop initially on muskmelons planted in an adjacent newly cleared field. In this experiment both fields were planted with the same lot of treated seeds. Dissemination of conidia eventually spread the disease to the muskmelons planted on the virgin land. Additional information was afforded by an experiment in which muskmelon seeds were infested with dry conidia, stored over the winter, and planted in the spring on land with no previous history of the occurrence of the disease. The resulting crop became heavily infected, suggesting that the seed borne conidia were primarily responsible for initiating the disease. Middleton and Whitaker (37) believed that the infection of the crown leaves of muskmelon, commonly observed by them, suggested that the inoculum was borne on the seeds.

Since there was a lack of specific information concerning the ultimate sources of inoculum, experiments were undertaken to investigate the ways in which the fungus survives from season to season. Heavily infected muskmelon leaves were selected from a naturally infected planting and several leaves were securely held together in a wire screen packet

made by folding the edges of an 8 inch square of 1/4 inch hardware cloth. Some packets containing selected diseased leaves were placed on the surface of the soil in a garden plot while others were buried at depths of 6 and 9 inches. The packets were set out in the month of June and unearthed 8 1/2 months later. Small compacted masses of decayed leaf fragments were found in the packets which had been buried at 6 and 9 inches. Small fragments of individual leaf blades and portions of petioles were recovered from the surface packets. The soil and leaf fragments from several packets from each location were mixed with 500 ml of water for 15 seconds in a Waring Blender. The resulting supernatant suspension of plant debris and fine soil particles was decanted and used to inoculate 26 mature Rio Gold cantaloupe plants and 35 Black Diamond watermelon seedlings in the greenhouse. Although conditions for the development of the disease were very favorable, no infection occurred. The procedure was repeated 7 days later with the same result.

The soil and plant debris from additional packets were examined in several ways for the presence of conidia. Supernatant suspensions of the contents of individual packets from each location were examined directly, and after the addition of mineral oil. Leaf fragments were carefully examined for the presence of conidia. Using these methods, a single conidium was observed in the course of the examination of the contents of 12 packets.

The leaf fragments were washed in distilled water and placed in petri dishes on moistened filter paper or on the surface of V-8 agar. After 60 hours in these moisture chambers, conidia, typical of the Alternaria leafspot pathogen, were observed along the edges of the leaf fragments. Single-conidial isolations were made and the conidia resulting from these

cultures proved to be characteristically similar to the conidia of A. cucumerina. A further verification of the identity of the conidia was made by using them as inoculum. Mature cantaloupe plants and watermelon seedlings were inoculated with a suspension of conidia from these cultures. Initial symptoms of the Alternaria leafspot disease appeared on the watermelon cotyledons within 72 hours.

The almost complete absence of conidia on the decayed leaf tissue and in the soil surrounding the leaf fragments strongly suggests that conidia were not able to exist in these environments and that they probably do not have an important function in the over-wintering of the fungus as it occurs in plant debris in the field. As the fungus has been shown to persist in decayed leaf material, it is probable that the primary conidial inoculum results from the renewed growth of the mycelium in host debris when field conditions become favorable, and the subsequent production of conidia by this mycelium.

These conclusions have been partially substantiated by inspection, during March, of a field in which Alternaria leafspot had been prevalent during the previous fall season. Although the field had been plowed and planted with oats, fragments of muskmelon vines were found which were covered with a felty black fungal growth. Conidia were not present on this debris when it was collected. The infested plant remains were placed in a moisture chamber, and after 36 hours numerous conidia of several fungi were found. Two species of Alternaria were observed, the most abundant species resembling A. tenuis auct. sensu str. Conidia which were characteristic of the Alternaria leafspot pathogen were also present. Several of the latter type conidia were transferred to V-8 agar and allowed to grow and sporulate. Inoculation of watermelon and muskmelon seedlings

with the conidia from these cultures resulted in the development of symptoms of the disease within 72 hours.

The introduction of the disease into a new area possibly occurs, as Van Halfern (61) and Middleton and Whitaker (37) have suggested, through the use of seeds which are infested with conidia of the pathogen. In an experiment designed to investigate the possibility of such a manner of disease initiation, Rio Gold cantaloupe seeds were sterilized in 1:1000 mercuric chloride for 10 minutes, rinsed in distilled water, and dried at room temperature. One-half of the seeds were then infested with conidia by shaking them on the surfaces of heavily sporulating cultures of the pathogen. The uninfested control seeds and the infested seeds were stored at room temperature for 4 months, and then planted in separate flats filled with autoclaved white quartz sand. An equal number of seeds was planted in each flat. Of the 140 seeds planted, 127 control seeds and 115 infested seeds germinated and grew. There was no evidence of root or hypocotyl infection in either group. After 3 weeks a few of the seedlings from infested seeds exhibited small brown lesions, predominantly at the tips of the cotyledons. The Alternaria leafspot fungus was recovered from 2 of 8 lesions examined. Seedlings from uninfested seeds did not become infected.

While very few of the seedlings grown from infested seed became infected, there was a definite indication that seed-borne conidia were capable of infecting the cotyledons, thus leading to a limited production of conidia. In addition to this possible manner of introduction of the disease into new areas, it was shown that the dead silicles of L. virginicum, a ubiquitous cruciferous weed in Florida, may support growth and sporulation of the pathogen. Dissemination of conidia by wind or blowing

rain from this plant, and possibly other weeds, and from infected cucurbits in nearby areas may be a method by which the fungus is introduced into new areas.

Climatic Conditions Favoring Natural Infection

Observations of several experimental plantings of cucurbits have been made over a period of 2 years. During this time 4 successive crops of muskmelons were killed or severely affected so that the quality of the fruits produced was poor. In addition, severe infection of Hubbard squash, Yellow Summer Crookneck squash, and Table Queen Acorn squash was noted during 1 growing period. The primary causes of the failure of these crops were, in 3 instances, Alternaria leafspot, and in 1 instance Alternaria leafspot and downy mildew. Climatological data, taken from a recording station within 200 yards of the experimental plots, were obtained for 2 of the 4 seasons during which infection and rapid disease development were noted. During September, 1956, initial infection of muskmelons occurred in the plots. The mean daily temperatures for this month were between 67° and 88°F and total rainfall, distributed among 12 days of the month, was 3.9 inches. In the second week of May, 1957, another severe attack of the disease was noted in its initial stage. Mean daily temperatures in May ranged between 63° and 85°F and total rainfall, distributed among 17 days, was 8.6 inches. Mean daily relative humidity during May fluctuated from a maximum of 99.5 per cent to a minimum of 42.7 per cent, with a mean daily duration of maximum relative humidity of 9.2 hours. These data indicate some of the ecological conditions under which the disease is initiated and develops. Unusually abundant rainfall and warm weather was noted by Chupp (9) to precede a severe attack of Alternaria leafspot of muskmelon in New York. Blinn (5) observed that the disease was always

severe during rainy seasons or when heavy dews occurred frequently. Judging from the report of Godfrey (18) who observed an epiphytotic outbreak on muskmelons during the dry season in Texas, an abundance of rainfall is not essential providing that moisture in some form is available at frequent intervals. Weber and Owen (65) observed heavy infection of watermelon in central Florida during a period marked by 30 per cent less rainfall than the previous 48-year average rainfall for the same months. The total amount of rainfall apparently is less important than the daily distribution of rainfall, dew, and high humidity.

Reports of field occurrences of the disease do not specify the temperature conditions prevailing at the time of infection. Chupp's (9) observation that the weather was unusually warm when the disease was noted gives only a general indication of temperature. Field observations have indicated that the disease occurred in severe proportions only when daily temperatures were in the range of 20° to 32°C (68° to 89.6°F). Low temperatures, which are common during February and March when many cucurbits are planted in Florida, may prevent infection of seedlings, enabling the crop to escape infection until higher temperatures occur.

Host Penetration

Penetration of the host by germination hyphae of the fungus was studied in a manner detailed by Diener (12), which employed cleared discs of whole leaves stained with 1 per cent cotton blue in lacto-phenol. The host leaf epidermis was penetrated directly by the germination hyphae of the fungus. Conidia germinated and produced 1-5 germination hyphae which grew rapidly over the surface of the leaf. An enlargement of the terminal portion of the hypha occurred prior to direct penetration of the leaf surface. Further elongation of the hypha was initiated from the lower

distal end of the enlargement or occasionally from a lateral position on the enlarged portion of the hypha. Penetration of the host occurred at this time, as shown in Figure 13, or after the formation of additional terminal enlargements. Penetration through open stomata was not observed, although many hyphae were observed to have grown directly over stomata.

Pathological Anatomy

Infected leaf material used for the following observations was obtained by inoculating the adaxial surfaces of Rio Gold cantaloupe leaves with suspensions of conidia from several isolates. Paraffin sections of lesions were prepared and stained with safranin and fast green in a manner prescribed by Riker and Riker (51).

Examination of serial sections of young lesions revealed that after penetration, hyphae branched and grew in a radial and downward manner, proceeding intercellularly and intracellularly. Their downward growth, while not progressively observed, occurred at an early stage since the abaxial diameters of the lesions were essentially the same as the adaxial diameters at all stages of development. The advance of the fungus into adjacent uninfected tissue usually occurred by intercellular growth of the hyphae between the adaxial epidermal cells and the palisade parenchyma, and concomitantly, between the abaxial epidermal cells and the spongy parenchyma. The rate of hyphal growth was slightly greater beneath the adaxial epidermis. The intercellular hyphae penetrated the palisade parenchyma cells at their adaxial ends and eventually destroyed cell contents and many of the cell walls, leaving small cavities and an irregular network of cell wall fragments. Penetration of the spongy parenchyma cells from beneath the abaxial epidermis and subsequent intracellular growth of the hyphae resulted in destruction of cell contents and most of the cell walls

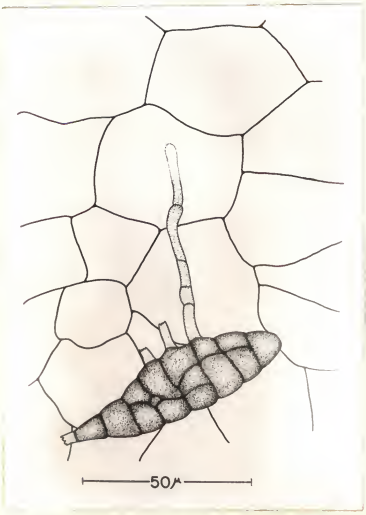

Fig. 13.--Camera lucida drawing of the penetration of an epidermal cell by a germination hypha of the fungus. Entire lengths of hyphae at left and conidial beak are not shown.

and caused a gradual collapse of this tissue. In each instance the epidermal tissue was invaded also.

In a transverse section of a young lesion, as shown in Figure 14, the intracellular growth of the fungus in the palisade parenchyma cells can be seen clearly. Several hyphae have become arranged within each cell, parallel to the long axis of the cell. The destruction of the palisade parenchyma has been relatively greater than that of the spongy parenchyma at this stage. This difference is believed to be due to the time of infection of these tissues in this central region of the lesion, since downward growth of the hyphae necessarily preceded infection of the spongy parenchyma. Differences in resistance to infection have not been observed among the leaf tissues. The average thickness of the infected portion of the leaf tissues shown in the upper portion of Figure 13 was 255μ. The thicknesses of the uninfected interveinal portions of the leaf averaged 280μ showing that the infected area was slightly depressed at this early stage of disease development.

As growth of the fungus proceeded radially outward from the point of initial penetration, the cell wall fragments in the area of initial infection collapsed to form a more compact mass of necrotic tissue. This collapse was more pronounced in the spongy parenchyma tissue and resulted in a greater depression of the lesion on the abaxial side. Average thickness of the necrotic tissue in the older lesion, shown in the lower portion of Figure 14, was 182μ, while adjacent uninfected tissue averaged 255μ. An abcission layer along the outer edges of the developing lesion was not observed.

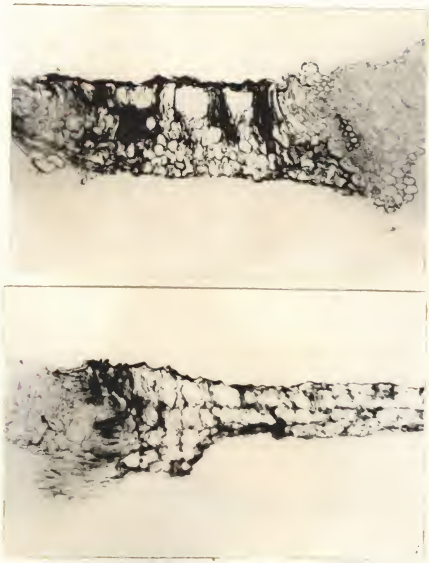

Fig. 14.--Photomicrographs of transverse sections of Rio Gold cantaloupe leaves made through the centers of lesions. The diameter of young lesion at top is 0.7 mm. The older lesion, the edge of which is shown, is 3.4 mm in diameter.

SUMMARY

1. Alternaria leafspot of cucurbits is a serious disease in the United States, causing considerable losses in areas where cucurbits are grown commercially.
2. Muskmelon is the principle host of the pathogen. Other hosts include watermelon, cucumber, 3 commercially grown species of squash, bur gherkin, balsam apple, balsam pear, and Melothria pendula.
3. Symptoms of the disease on muskmelon, watermelon, cucumber, and squash are described in detail.
4. The valid name of the causal organism is Alternaria cucumerina (E. & E.) J. A. Elliot, and it is proposed that A. brassicae f. nigrescens Pegl., A. nigrescens (Pegl.) Neerg., and Macrosporium cucumerinum E. & E. be regarded as invalid synonyms.
5. An emendation to the present descriptions of the fungus is proposed.
6. Greatest germination of conidia occurs between 20° and 28°C.
7. The rates of mycelial growth of 3 isolates in liquid medium are different but the isolates show the same relative responses to given temperatures.
8. Greatest growth of mycelium occurs at temperatures of 24° to 28°C.
9. Growth of mycelium is greater at pH 5.0 than at higher or lower pH values tested.
10. Temperature and pH affect the rate of mycelial growth independently.
11. Dormant mycelium in plant debris resumes growth when ecological conditions become favorable and conidia produced by this mycelium serve as inoculum for initial spring infections.
12. Conidia do not survive in a soil environment in great enough numbers

to serve as initial spring inoculum.

13. Infection of cucurbits and subsequent infestation of the soil in disease-free areas is believed to be caused by seed-borne and wind-disseminated conidia.

14. Host penetration and pathological anatomy are described.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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